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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
GOLDBERG, JEANINE ANNE	
ART UNIT	**PAPER NUMBER**
1634	

DATE MAILED: 03/21/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/872,493	KENNY ET AL.	
	Examiner	Art Unit	
	Jeanine A. Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 February 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-12 and 14-35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-12 and 14-35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is in response to the papers filed December 14, 2004 and February 28, 2005. Currently, claims 1, 3-12, 14-35 are pending. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow.

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 14, 2004 has been entered.

3. The claims have been amended to require that the nucleic acid analyte is DNA, endogenous gene or segments thereof. It is clear from the specification which is directed to endogenous gene transcripts that endogenous genes does not encompass RNA. The claims have been specifically amended to exclude RNA. Moreover the declaration under 132 and the arguments are directed at distinguishing the prior art directed to RNA from DNA of the instant claims.

Priority

4. This application claims priority to provisional application 60/209,139, filed June 2, 2000.

Drawings

5. The drawings are acceptable.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

7. Claims 1, 3-4, 6-23, 27-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Antao et al. (Techniques in Quantification and localization of gene expression, page 81-93, June 1999) in view of Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Antao et al. (herein referred to as Antao) teaches a method for in situ detection of a nucleic acid analyte within a sample based on bDNA hybridization by a) preparing the sample by immobilizing, permeabilizing using proteinase K b) contacting with a probe wherein at least a portion of the target probe is complementary to at least a portion of

the nucleic acid analyte c) washing with a detergent at a temperature of approximately 21 to 60 C (i.e. room temperature) and d) detecting the analyte-target probe complex on the substrate (Figure 6.2, page 84)(limitations of Claim 1). Specifically, Antao teaches fixing cells to a slide (page 84-95)(limitations of Claim 20). Antao teaches using a number of cell lines and cell strains including lymphoid cell line, blood cells (limitations of Claims 21-22, 32-33). The microscope slides are incubated in proteinase K (0.5 ug/ml in PBS)(page 85)(limitations of Claim 4). The slides are prepared for prehybridization by applying diluent A at 65 degrees C (page 85)(limitations of Claim 1aiii). Diluent A comprises 6X SSC, 25% formamide, detergent and casein. The increased temperature to 65C for 30 minutes in combination with the formamide would denature dsDNA or remove RNA secondary structure. The slides are then contacted with target probes (page 86). The slides are rinsed in wash buffer at room temperature, i.e. between 21 and 60 degrees Celsius (page 86)(limitations of 1c). The hybridized probe is then detected using label probe with a fluorophore directly attached to it or is conjugated to an AP or horseradish peroxidase molecule (page 86)(limitations of Claims 16-17). Antao further teaches incubating the slides with amplifier. As seen in Figure 6.2, the preamplifier is complementary to a portion of the target probe and forms an analyte-target probe-preamplifier probe complex. Also, an amplifier is hybridized which is complementary to the preamplifier. Moreover, a Ap, fluorescent or chromagenic label is added to the amplifier probe and the presence of the label is detected. Antao teaches detecting two targets, HIV-1 viral RNA and hn RNP A2/B1 mRNA (limitation of Claim 3). The method of bDNA technology for *in situ* detection which allows for quantification and is specific, reproducible, easy to use and yields results within one day (page 83). Antao

teaches using 50ul of preamplifier and amplifier at 0.4 fmol/ul, namely using 20 fmol of preamplifier and amplifier (limitations of Claims 14-15). Antao teaches using 50ul of 2-6 fmol/ul of target probe, namely 0.1-0.3 pmol of target (limitations of Claim 6). The washing solution is comprised of Tris, MgCl2, detergent and ZnCl2 (limitations of Claims 9-10). Antao teaches washing the slide at least twice (page 86)(limitations of Claims 11-12). Each of the wash steps are carried out a room temperature (limitations of Claim 13). Antao teaches centrifuging at 1,500 rpm for 6 minutes (page 85)(limitations of Claim 23).

Antao does not specifically teach washing the biological material with a detergent.

However, Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that 0.1-0.5 ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10). Xu teaches that in some protocols, a higher concentration of detergent is used (1% Triton X-100) to give a cleaner background but can substantially reduce the signal (page 96). Additionally, Xu teaches in Protocol 2, the treatment with proteinase K followed by prehybridization with hybridization solution and incubation at 55-65C. The hybridization solution further comprises 50% formamide

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the known bDNA hybridization assay of Antao which does not specifically provide for a detergent in the wash with the wash solution of Xu which comprises Triton. The ordinary artisan would have been motivated to have used the detergent for the expected benefit taught in the art by Xu, so as to reduce the background. Although Xu also teaches that the signal may be reduced, the method of Antao is specifically designed for signal amplification, therefore, the ordinary artisan would not anticipate this would cause any negative effects on the detection. The ordinary artisan would have a reasonable expectation of success for using a detergent in the in situ method of Antao to reduce background signal and ensure the signal being amplified by Antao is in fact the signal desired and not background signal. Therefore, the ordinary artisan would have used a wash solution with detergent for the expected benefits taught in the art.

Response to Arguments

The response and declaration filed on February 28, 2005 provides evidence to indicate that the Antao reference publication date was January 21, 2000 and not June 1999. The response indicates that the publisher of the book was contacted, as provided in Appendix A. Since the reference qualifies under 102(a), a declaration under 1.131 was submitted, unexecuted, to antedate the Antao reference. Had the declaration been executed by each of the inventors, the rejections relying on Antao would be withdrawn.

Thus for the reasons above and those already of record, the rejection is maintained.

8. Claims 1, 3-12, 16-17, 20-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moraes et al. (Methods in Enzymology, Vol. 264, pages 522-540, 1996) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) in view of in view of Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Moraes et al (herein referred to as Moraes) teaches a method for detection and analysis of mitochondrial DNA and RNA in muscle by in situ hybridization. Moraes teaches ISH has provided a wealth of information on the intercellular distribution of heteroplasmic mitochondrial DNA (mtDNA) populations (abstract). Moraes teaches glass slides should be treated with polylysine to ensure adherence of the sample to the glass (page 523, para 2)(limitations of Claim 4). Moraes teaches preparing muscle sections comprising cells (limitations of Claim 20, 21, 22, 24-26). Moraes teaches that sections are treated with proteinase K (5 ug/ml in 1X PBS)(page 524, number 3)(limitations of Claim 5). Moraes teaches the protocol for preparation of muscle sections includes these distinctions for in situ hybridization differences between RNA and DNA. Specifically, the protocol states that "if detection of mtRNA is desired, proceed to step 10 without further treatment." This skipping of steps includes the skipping of the treatment with RNase. Specifically, step 8 requires that "for detection of mtDNA, samples should be treated with Dnase-free Rnase." (Page 524). Further Moraes teaches that when performing RNA detection, the denaturation step is omitted (page 525, item 12). Moraes outlines the protocol for in situ hybridization with DNA

probes (page 525-526). Moraes also outlines the protocol for in situ hybridization with RNA probes (page 527-529). Moraes teaches washing with 2X SSC at room temperature for 1 hour (page 526). Moraes also teaches washing at 0.1X SSC, 14.4 mM 2-mercaptoethanol, and 0.05% w/v sodium pyrophosphate at 50 degrees. Moraes teaches the washing solution should be kept at 50 degrees with gentle stirring (page 526, item 4)(limitations of Claim 11). As seen in the graph provided on page 530, the quantity of mRNA and mtDNA are both analyzed. It is clear that both DNA and RNA are detected using the protocols. Figure 2 also illustrates the cellular localization of mtDNA by in situ hybridization (page 531)(limitations of Claim 27). Moraes teaches separate protocols for the DNA and RNA hybridization. The ordinary artisan would have recognized that minor modifications would differ between the protocols for DNA and RNA detection. However, the art teaches detection of both DNA and RNA, therefore, it would have been within the skill in the art to optimize and detect either RNA or DNA, as taught by Moraes.

Moraes does not specifically teach the use of bDNA for amplification of signals.

However, Cao teaches that development of a non-isotopic in situ hybridization technique for detection of nucleic acid in archived sputum specimens using branched DNA technology (in situ bDNA) is successful.

Further, Nolte teaches branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Nolte emphasizes that the number of target molecules is not altered in the method of bDNA and the resulting signal is directly proportional to the concentration of the target nucleic acid (page 202). The signal of direct hybridization rather than the nucleic acid itself is amplified and thus is directly

proportional to the amount of target sequence present in the clinical sample (page 214). BDNA is a nonenzymatic process and is less prone to sample-to-sample variation than with enzymatically mediated target amplification systems. Figure 1 illustrates the bDNA signal amplification assay (limitations of Claims 2). Nolte describes comparisons which were performed between various assays. Nolte teaches that as study by Butterworth teaches the bDNA assay was linear over three orders of magnitude and was the most sensitive assay being approximately 10 times more sensitive than the other assays. Butterworth teaches that the amplifier oligonucleotides enhance the chemiluminescent signal by having multiple branches of the same sequence which bind to the alkaline phosphatase labeled probes which reacts with Dioxetane to produce a chemiluminescent signal which is detected by a luminometer and the concentration of HBV-DNA is determined (limitations of Claim 16, 17). Nolte teaches using bDNA to detect HCV, for example (page 206)(limitations of Claim 3). Nolte suggests the application for bDNA in *in situ* hybridization assays (page 231).

Schaeren-Wiemers et al. (herein referred to as Schaeren) teaches *in situ* hybridization using digoxigenin-labelled cRNA probes. Schaeren teaches *in situ* hybridization methods for tissue sections and cultured cells using digoxigenin-labelled cRNA probes (abstract). Schaeren teaches mounting on a polylysine coated slide (page 433)(limitations of Claim 4, 20). Schaeren teaches washing in 5xSSC and 0.2 X SSC (page 433, col. 2)(limitations of Claim 11). Schaeren teaches using tissue sections, namely brains and cell cultures, namely optic nerves (page 433, col. 2)(limitations of Claim 21-22, 24-26). Schaeren teaches culturing cells, aspirating off the medium, thereby having used a centrifuge (limitations of Claim 23).

Moreover, Decimo et al. provides basic conditions for conducting *in situ* hybridization assays. Decimo teaches pre-hybridization treatments of slides with proteinase K, 1 ug/ml (page 192). The hybridization is performed followed by washing steps. The post-hybridization washes were performed at 55 C for about 45 minutes (limitations of Claim 1c, 13). Decimo teaches that approximately 5-10 ul of hybridization mixture is required per cm2 of tissue section. Decimo teaches several wash steps following hybridization (page 195)(limitations of Claim 11-12).

Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Specifically, Xu teaches in Protocol 2, the treatment with proteinase K followed by prehybridization with hybridization solution and incubation at 55-65C. The hybridization solution further comprises 50% formamide. Therefore, under the conditions presented by Xu, the heating the proteinase K treated cells to 55-65 C in 50% formamide would denature double stranded DNA and remove RNA secondary structure. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that 0.1-0.5 ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10). Xu teaches that in some protocols, a higher concentration of detergent is used (1% Triton X-100) to give a cleaner background but can substantially reduce the signal (page 96).

With respect to Claim 6, Schaeren does not specifically teach using approximately 0.1 pmoles to 10 pmoles of the target probe. Schaeren teaches

preparing hybridization mixture by adding 200 ng cRNA per ml hybridization buffer. Moreover, 200 ul hybridization buffer was used. However, Xu teaches average *in situ* probes are between 32-36 nucleotides in length. Thus, using a 34 nucleotide probe as an average length, 130 as the average molecular weight of each base, the probe is approximately 4,420 g/mol. Thus, there is approximately .91 pmoles of target probe used. Thus, performing the *in situ* hybridization with various length probes, probes of various sequences, would have been within the optimization of the ordinary artisan. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

Claim 27 requires that the position of a nucleic acid analyte within a sample is determined as indicative of the position of the nucleic acid analyte in the cell. Thus, the mere detection of the nucleic acid within the cell would be indicative of the position of the nucleic acid within the cell. Thus, based upon the language of the claim, the claim only requires identifying the complex within a cell.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the *in situ* hybridization assay using labelled DNA and RNA probes as taught by Moraes with the improved bDNA hybridization detection method. The prior art teaches that ISH has been used to detect both DNA and RNA in cells and the localiziation (see Moraes). The prior art teaches protocols for detecting DNA and RNA in situ which vary with minor modifications. Thus, the prior art teaches that DNA may be routinely detected in situ. The prior art also teaches that branched DNA technology is used in situ for detecting mRNA, as taught by Nolte, to be more sensitive, precise and linear. Since the prior art teaches the ordinary

artisan how to modify in situ protocols to detect both DNA and RNA in a given cell, the ordinary artisan would have a reasonable expectation of success for using bDNA in situ to detect DNA. The ordinary artisan would have been motivated to have detected DNA using bDNA because the quantity of DNA within a cell is smaller, see Moraes. Given the comparison of solution hybridization methods taught by Nolte, there would have been a reasonable expectation that the bDNA method would have been more sensitive, precise and linear *in situ*. Hybridization mechanisms *in situ* and in solution are analogous, thus, the ordinary artisan would have expected bDNA to act similarly in DNA and RNA. *In situ* hybridization detection of probes would be enhanced by signal amplification such as bDNA. The signal amplification would allow for greater luminescence and greater detection signals.

With respect to the specific conditions for performing *in situ* hybridization, the skilled artisan would have been motivated to have optimized the conditions. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the probe selection performed was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. Thus, performing the *in situ* hybridization with conditions provided by Decimo as optimal for ISH, would have been within the optimization of the ordinary artisan. Moreover, Xu provides specific motivation why to perform the method using a detergent in the wash step. Therefore, using particular

concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

Response to Arguments

The response traverses the rejection. The response's arguments are directed to the claims prior to the amendment to require detection of DNA, not RNA. Thus, in view of the amendments to the claims, the rejection above is a new grounds of rejection necessitated by amendment.

The declaration filed by Daryn Kenny on December 14, 2004 has been thoroughly considered. The declaration directs the examiner's attention to a detailed discussion of the problems previously encountered in bDNA in situ hybridization assays and the advantages that the present invention has had in overcoming the previous problems. Daryn Kenny submits the J. Histochem. Cytochem. Article for review. In the review, Kenny et al state the protocol for pretreatment of tissue sections for DNA. Kenny then states that "for detection of mRNA, the procedure was the same except that there was no RNAase digestion or high-temperature denaturation." This appears to be the difference in protocol between DNA and RNA in situ detection. The declaration is silent with respect to any additional differences or differences which are required by the instant claims.

Turning to Moraes, the protocol for preparation of muscle sections includes these distinctions for in situ hybridization differences between RNA and DNA. Specifically, the protocol states that "if detection of mtRNA is desired, proceed to step 10 without further treatment." This skipping of steps includes the skipping of the treatment with RNase. Specifically, step 8 requires that "for detection of mtDNA, samples should be treated

with Dnase-free Rnase." Thus, the first difference between RNA and DNA detection was taught in the prior art as a known difference for detecting RNA vs. DNA. Further Moraes teaches that when performing RNA detection, the denaturation step is omitted (page 525, item 12). It is noted that Claim 1 requires this "optional" step. Thus, the two differences in protocol between the DNA and RNA in situ detection appear to be taught in the art. Therefore, these minor modifications in protocol between RNA and DNA would have been obvious given the express teachings in the art.

The declaration also argues that when the inventors of the application applied the reaction conditions for RNA in situ hybridization using bDNA for signal amplification....the results were not satisfactory (point 8). The background noise in the reactions was such that DNA signals could not be seen. The declaration further asserts that "because of the inability of the RNA assays described in Antao et al. and Xu et al. to provide adequate use of a bDNA in situ assay for the detection of DNA" steps were taken to correct the deficiencies in the prior art. As provided in MPEP 716.01(c), "Objective evidence which must be factually supported by an appropriate affidavit or declaration to be of probative value includes evidence of unexpected results, commercial success, solution of a long-felt need, inoperability of the prior art, invention before the date of the reference, and allegations that the author(s) of the prior art derived the disclosed subject matter from the applicant. See, for example, In re De Blauwe, 736 F.2d 699, 705, 222 USPQ 191, 196 (Fed. Cir. 1984) ("It is well settled that unexpected results must be established by factual evidence." "[A]ppellants have not presented any experimental data showing that prior heat-shrinkable articles split. Due to the absence of tests comparing appellant's heat shrinkable articles with those of the

closest prior art, we conclude that appellant's assertions of unexpected results constitute mere argument."). See also In re Lindner, 457 F.2d 506, 508, 173 USPQ 356, 358 (CCPA 1972); Ex parte George, 21 USPQ2d 1058 (Bd. Pat. App. & Inter. 1991)." Here the arguments directed to the results were not satisfactory and background noise was such that DNA signals could not be seen does not constitute evidence. The declaration appears to assert an opinion of the inventor which is given weight, but is unable to overcome the teachings of obviousness since there is no evidence to support the arguments. The declaration has not supported the arguments by evidence such as comparative to the closest prior art. The MPEP provides that "Although an affidavit or declaration which states only conclusions may have some probative value, such an affidavit or declaration may have little weight when considered in light of all the evidence of record in the application. In re Brandstadter, 484 F.2d 1395, 179 USPQ 286 (CCPA 1973).

Further, it is noted that the claims are not limited to any of the differences between the stated DNA and RNA protocols. Thus, the claims do not appear to contain the required differences between the DNA and RNA protocols.

With regard to Claim 27, Moraes teaches and illustrates cellular localization of mtDNA by in situ hybridization (see Figure 2).

Thus for the reasons above and those already of record, the rejection is maintained.

9. Claims 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over

Moraes et al. (Methods in Enzymology, Vol. 264, pages 522-540, 1996) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) in view of in view of Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999) as applied to claims 1, 3-12, 16-17, 20-27 above and in further view of Kern et al. (J. Clin. Microbiol. Vol. 34, No. 12, pages 3196-3202, 1996).

Neither Moraes, Schaeren, Cao, Nolte, Decimo, nor Xu specifically teaches using between 1 fmol and 10pmoles of preamplifier or amplifier.

Kern teaches a method of bDNA quantification of HIV. The method of kern teaches using .70 fmol of preamplifier per ul and 1.0 fmol of bDNA amplifier per ul. The 50ul of solution was used.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have used the specified conditions provided by Kern in the absence of specific conditions provided in Nolte for the amount of preamplifier and amplifier used in a bDNA assay. The ordinary artisan would have looked to the art to the ranges and units of preamplifier and amplifier used in bDNA assays to optimize the method of using bDNA and in situ hybridization. Thus, using approximately 1 fmol to about 10pmoles of amplifier and of preamplifier, would have been obvious to the ordinary artisan at the time the invention was made.

Response to Arguments

The response traverses the rejection. The response asserts that because the teachings of Moraes, Schaeren-Weimers in view of Cao, Nolte, Decimo and Xu do not

render the claimed invention obvious, the additional teachings will not serve to render Claim 14-15 obvious. This argument has been thoroughly reviewed, but is not found persuasive for the reasons of record above. Thus for the reasons above and those already of record, the rejection is maintained.

10. Claims 18-19, 28-35 are rejected under 35 U.S.C. 103(a) as being unpatentable Over Moraes et al. (Methods in Enzymology, Vol. 264, pages 522-540, 1996) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) in view of in view of Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999) as applied to claims 1, 3-12, 16-17, 20-27 above and in further view of and further in view of Plummer et al. (Diagnostic Mol. Pathology, Vol. 7, No. 2, pages 76-84, 1998).

Neither Moraes, Schaeren, Cao, Nolte, Decimo, nor Xu specifically teach the method has a sensitivity to detect from 1-2 copies of the nucleic acid.

However, Plummer teaches a method of detection of low copy number nucleic acid sequences using catalyzed reporter deposition. Plummer teaches detection of HPV in SiHa cells with 1-2 copies of HPV 16. The CARD-ISH system detected one to two copies of HPV 16 in the SiHa cells whereas the conventional ISH method did not. Plummer teaches that the catalyzed reporter deposition (CARD) technique has been shown to enhance the detection of low copy DNA and RNA sequence in formalin-fixed paraffin-embedded sections. CARD was developed as a signal amplification system. Plummer teaches the components of the CARD-ISH technique. Plummer teaches that

the CARD-ISH using oligonucleotides probes was examined to explore the sensitivity of the system for alternative DNA and RNA target sequences.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have performed the *in situ* hybridization method of Schaeren in view of Nolte with the optimized conditions of Decimo or Xu and further have optimized the *in situ* hybridization assay to detect a single copy of the nucleic acid analyte in a cell, as taught by Plummer. Plummer teaches the importance of detecting single copy nucleic acids and provides means by which the *in situ* hybridization may be performed. Plummer specifically teaches that detection of low copy number in cells is feasible by using the signal amplification method of CARD. The ordinary artisan would have had a reasonable expectation of success that the signal amplification method of bDNA would similarly allow the detection of low copy numbers of DNA. Since Plummer teaches that signal amplification allows for the detection of low copies of DNA without the requirement of specialized or expensive equipment, allows the entire procedure to be rapidly completed and does not create additional health risks (page 83 of Plummer). The ordinary artisan would have been motivated to have optimized the in situ hybridization assay to include detection of single copy targets.

11. Claims 28-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Plummer et al. (Diagnostic Mol. Pathology, Vol. 7, No. 2, pages 76-84, 1998) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Plummer teaches a method of detection of low copy number nucleic acid sequences using catalyzed reporter deposition. Plummer teaches detection of HPV in SiHa cells with 1-2 copies of HPV 16. The CARD-ISH system detected one to two copies of HPV 16 in the SiHa cells whereas the conventional ISH method did not. Plummer teaches that the catalyzed reporter deposition (CARD) technique has been shown to enhance the detection of low copy DNA and RNA sequence in formalin-fixed paraffin-embedded sections. CARD was developed as a signal amplification system. Plummer teaches the components of the CARD-ISH technique. Plummer teaches that the CARD-ISH using oligonucleotides probes was examined to explore the sensitivity of the system for alternative DNA and RNA target sequences.

Plummer does not specifically teach using bDNA for signal amplification and detection of nucleic acid analytes *in situ*.

However, Cao teaches that development of a non-isotopic in situ hybridization technique for detection of nucleic acid in archived sputum specimens using branched DNA technology (in situ bDNA) is successful.

Moreover, Nolte teaches branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Nolte emphasizes that the number of target molecules is not altered in the method of bDNA and the resulting signal is directly proportional to the concentration of the target nucleic acid (page 202). The signal of direct hybridization rather than the nucleic acid itself is amplified and thus is directly proportional to the amount of target sequence present in the clinical sample (page 214). BDNA is a nonenzymatic process and is less prone to sample-to-sample variation than with enzymatically mediated target amplification systems. Figure 1

illustrates the bDNA signal amplification assay (limitations of Claims 2). Nolte describes comparisons which were performed between various assays. Nolte teaches that as study by Butterworth teaches the bDNA assay was linear over three orders of magnitude and was the most sensitive assay being approximately 10 times more sensitive than the other assays. Butterworth teaches that the amplifier oligonucleotides enhance the chemiluminescent signal by having multiple branches of the same sequence which bind to the alkaline phosphatase labeled probes which reacts with Dioxetane to produce a chemiluminescent signal which is detected by a luminometer and the concentration of HBV-DNA is determined (limitations of Claim 16, 17). Nolte teaches using bDNA to detect HCV, for example (page 206)(limitations of Claim 3). Nolte suggests the application for bDNA in *in situ* hybridization assays (page 231).

Moreover, Decimo et al. provides basic conditions for conducting *in situ* hybridization assays. Decimo teaches pre-hybridization treatments of slides with proteinase K, 1 ug/ml (page 192). The hybridization is performed followed by washing steps. The post-hybridization washes were performed at 55 C for about 45 minutes (limitations of Claim 1cm 13). Decimo teaches that approximately 5-10 ul of hybridization mixture is required per cm2 of tissue section. Decimo teaches several wash steps following hybridization (page 195)(limitations of Claim 11-12).

Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that 0.1-0.5

ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10). Xu teaches that in some protocols, a higher concentration of detergent is used (1% Triton X-100) to give a cleaner background but can substantially reduce the signal (page 96).

With respect to Claim 35, depending upon the sample available the ordinary artisan would have been motivated to have used tissue samples as opposed to cells for analyzing the nucleic acids *in situ*. Therefore, using the methods taught in the art for preparation of tissue samples, would have been obvious to the skilled artisan at the time the invention was made. With specific respect to the tissue types recited in Claim 35, the skilled artisan would have been motivated to have used any of the tissue types provided depending upon the sample available for analyses. In situ hybridization has been used on biopsies from cancer patients, thus, any tissue biopsy which requires analyzation would be appropriate and the ordinary artisan would be motivated to analyze the biopsy tissue.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the *in situ* hybridization assay using DNA oligonucleotide probes and signal amplification with the improved bDNA signal amplification hybridization method. The ordinary artisan would have been motivated to have modified the signal amplification CARD method, as taught by Plummer, with the bDNA signal amplification method. There would have been a reasonable expectation that the bDNA method would have been more sensitive, precise and linear in situ. *In situ* hybridization detection of probes would be enhanced by signal amplification such as bDNA. The signal amplification would allow for greater luminescence and greater

detection signals. Thus, the ordinary artisan would have been motivated to have used the signal amplification method of bDNA in the signal amplification method taught by Plummer because the skilled artisan would have recognized that the bDNA signal amplification method is a quantitative method which is very sensitive.

Conclusion

12. No claims allowable over the art.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (571) 272-0743. The examiner can normally be reached Monday-Friday from 7:00 a.m. to 4:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571) 272- 0745.

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The Central Fax Number for official correspondence is (571) 273-8300.

J Goldberg

Jeanine Goldberg
Primary Examiner
March 17, 2005